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TITLE: Transient Delivery of Adenosine as a Novel Therapy to Prevent Epileptogenesis

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14. ABSTRACT <p>Epigenetic changes, including hypermethylation of DNA, are fundamental to progression and maintenance of epilepsy. Using silk-based brain implants engineered to release adenosine we demonstrated that reversal of epigenetic changes prevents epileptogenesis. We identified a novel mechanism by which adenosine reduces DNA methylation in the brain and translated those findings into a new therapeutic strategy (biodegradable silk-based brain implants) to prevent epileptogenesis long term. These findings constitute a novel scientific advance with direct clinical implications. Specifically, using bioengineered silk-based brain implants we demonstrated that transient delivery of a defined focal dose of adenosine to epileptic rats can reverse pathological DNA hypermethylation. Further, we showed that this treatment can prevent epileptogenesis as assessed by the analysis of two independent outcome parameters (seizures and mossy fiber sprouting). To our knowledge this is the first study where a robust antiepileptogenic effect has been demonstrated <i>after</i> the onset of epilepsy. Adenosine and silk are FDA approved; thus our findings have direct translational value. In summary, we demonstrated that DNA methylation changes are integral to initiation and progression of epilepsy; these epigenetic changes are modulated by adenosine, which is dysregulated in epilepsy; focal transient silk-based adenosine augmentation reduces epilepsy associated DNA hypermethylation and halts disease progression.</p>					
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Introduction

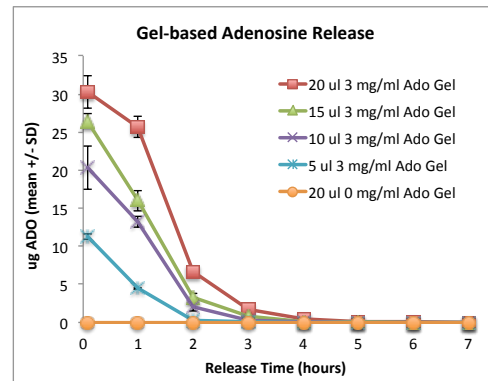
Epilepsy is a progressive neurological disorder; even with optimal treatment, ~35% of patients develop pharmacoresistant seizures, leaving them with limited treatment options and poor quality of life. Current drug treatments are designed for symptom control (i.e. seizure suppression) but do not affect the underlying pathophysiological mechanisms and do not prevent epileptogenesis. Adenosine is an endogenous network regulator of the brain with proven antiepileptic properties. Adenosine deficiency is a pathological hallmark of the epileptic brain and directly implicated in seizure generation and epileptogenesis. Importantly, transgenic animals with increased levels of adenosine in the brain are resistant to the development of epilepsy. The goal of this research is to develop a therapeutic approach to utilize adenosine for the prevention of epileptogenesis, which continues to remain an important goal to prevent epilepsy development in OIF and OEF veterans. Unfortunately, systemic adenosine augmentation is not a therapeutic option due to systemic side effects. We therefore selected to develop a silk-based brain implant to deliver a defined dose of adenosine for a predetermined period of time to a critical site in the brain. Our overarching goal is to use those adenosine-releasing silk-based brain implants to prevent epileptogenesis in a rat model of progressive epilepsy development. We will determine effective doses, potential side effects, and suitable time points and time frames of therapeutic intervention. Silk is a biodegradable biopolymer. If a silk-polymer can be used to prevent epileptogenesis through the transient delivery of adenosine it would be an ideal therapeutic application following an epileptogenesis triggering event such as TBI. Once its job to prevent epileptogenesis is done, the silk will be resorbed without leaving any residues. The *expected outcome* of this grant is the demonstration that a transient dose of adenosine can prevent epileptogenesis, to define the underlying mechanisms, and to determine suitable doses and time points of therapeutic intervention.

Body

The following sections describe our 2nd year progress in each of the Tasks:

Task 1: Silk Polymer Production:

We refined our silk polymer design to better control the adenosine release profile. A major refinement was to switch from a rigid silk-based brain implant that requires a major surgical intervention to a gel-based silk implant that can directly be injected into the target brain area. This gel-based format allows refinement of the delivery and improved reproducibility adenosine release. Adenosine delivery can be tailored both by volume of gel and by concentration of adenosine in the gel.

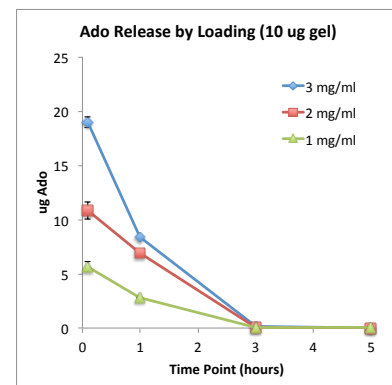


Overall conclusions:

- Silk in a gel format has greater reproducibility for acute delivery of adenosine.
- The gel format has improved delivery characteristics over the rigid polymer.

Ongoing Studies:

- In our *in vivo* experiments we have shown that a combination of an initial adenosine 'burst release' with the sustained delivery of a lower dose of adenosine over 10 days prevents epileptogenesis in our rat model. To assess whether the adenosine burst or the sustained low-dose delivery of adenosine is antiepileptogenic we need to separate out the two release components. We therefore compare 'burst release only' from adenosine releasing silk gels with 'sustained release only' from adenosine releasing osmotic pumps.
- Optimization of adenosine reservoir pellets with silk encapsulation for extended release options.



Task 1a: Efficacy & Toxicity:

Completed in Year 1; see Year 1 Progress Report

Task 1b: Differentiation:

Completed in Year 1; see Year 1 Progress Report

Overall conclusions:

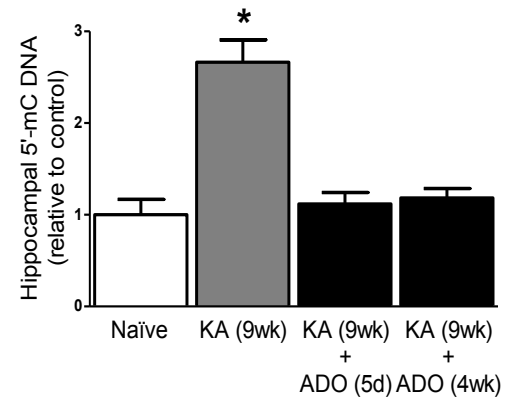
The effective dose range for chronic adenosine delivery is between 200 and 1000 ng silk-based adenosine per day; intracerebroventricular adenosine has no major side effects over a dose range from 50 to at least 3000 ng per day.

Task 2: Assess long-term impact of transient adenosine delivery in clinically relevant model of MTLE

Task 2a: Early Intervention:

Completed in Year 1; see Year 1 Progress Report.

Tissue samples from the experimental animals were further investigated to understand the underlying mechanism of antiepileptogenesis by transient silk-based adenosine delivery. We find global hypermethylation in epileptic hippocampus that is reversed with silk-based adenosine treatment. Our findings show that silk-based adenosine regulates DNA methylation as a putative target mechanism for epilepsy prevention.



Task 2b: Late Intervention:

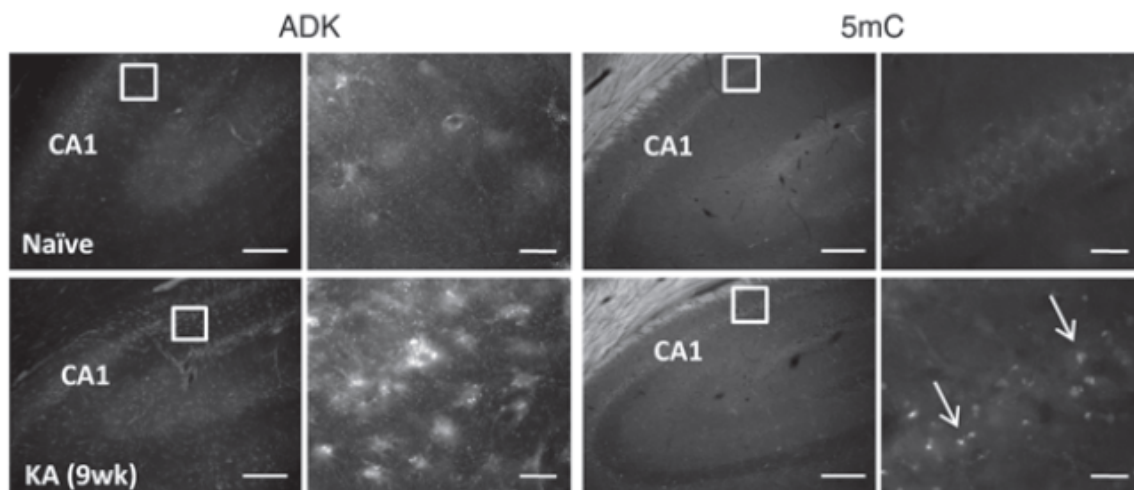
These are ongoing experiments that started in Year 2 and that extend into Year 3.

We presently have 30 post-kainic acid status epilepticus (post-KASE) rats in the colony; a total of 54 rats were treated with KA to achieve the necessary survival rate. Presently, rats are incubating and waiting for treatment (late intervention). Rats will be treated at 16 weeks post-KASE to demonstrate the efficacy of transient adenosine treatment during the chronic epilepsy.

To improve overall throughput and efficacy we have further optimized our animal model; administration of kainic acid in multiple smaller doses rather than a single bolus injection improved acute survival of the animals and increased the number of animals that develop status epilepticus.

Task 2d: Neuropathology:

Adenosine kinase (ADK) is a dominant regulator of adenosine homeostasis in the brain. In our prior work, we have demonstrated astrogliosis and ADK overexpression in several models of seizure and epilepsy. Seizures can be suppressed in these models by through pharmacologic activation of adenosine receptors, pharmacologic suppression of ADK activity, or by direct adenosine supplementation. In support of the global hippocampal methylation data presented above, we performed immunohistochemistry to examine the cellular distribution of methylation in the context of ADK overexpression in naïve and epileptic rats. As expected, we found increased ADK expression in the post-KASE rats, particularly in the CA1 region. We examined 5mC staining in serial sections, and found hypermethylation in the CA1 region as well, suggesting a potential link between the two phenomena, though we have not yet established the cause-effect relationship. The restoration of global methylation levels by adenosine augmentation suggests that the ADK overexpression drives the DNA hypermethylation.



Key Research Accomplishments

- Refinement of silk-based ADO delivery; we developed an adenosine releasing silk-based gel that can directly be injected into the brain.
- Development of a strategy to distinguish between effects of burst release vs. sustained release of adenosine.
- Identification of a mechanism by which adenosine prevents epileptogenesis. Identification of this mechanism will allow the development and exploitation of novel drug targets, such as DNA methylation inhibitors.
- We further optimized our animal model and implemented changes that reduced acute mortality and increased the rate of animals developing status epilepticus.

Reportable Outcomes

Manuscript:

Williams-Karnesky RL, Sandau US, Lusardi TA, Lytle NK, Farrell JM, Pritchard EM, Kaplan DL, Boison D (2013) Epigenetic changes induced by adenosine augmentation therapy prevent epileptogenesis. *Journal of Clinical Investigation* 123(8):3552-63.

Invited lectures since August 2013 (D. Boison):

59th Annual Meeting of the Radiation Research Society, New Orleans, LA

Symposium: CNS effects of radiation damage

"Prenatal radiation exposure – a risk factor for the development of epilepsy."

Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, PA

"Translational adenosine research."

Warwick University, School of Life Sciences, Coventry, UK

"Translational adenosine research."

Oregon Health and Science University, Portland, OR

Biology of Neurodegeneration (BOND) interest group

"Comorbidities in Neurology: the search for common mechanisms"

PAME (Partners Against Mortality in Epilepsy) Conference, Minneapolis, MN

Plenary Session: SUDEP Mechanisms: Respiratory

"Adenosine in Brainstem."

Purines 2014 (International Conference on Signaling, Drugs, and Targets), Bonn, Germany

Symposium: Adenosine deaminase and intracellular purine metabolizing enzymes

"Adenosine kinase: exploitation for therapeutic gain."

Conclusions

Acquired epilepsy is a progressive disorder, frequently manifesting many months or years after a precipitating brain injury such as TBI. The progression of epileptogenesis continues even after convulsive seizures begin to manifest, causing increased reliance on antiepileptic drugs and the development of drug resistance for up to 30% of patients. None of the currently used antiepileptic drugs affects the underlying pathogenetic mechanisms of epilepsy and none of those drugs halts or prevents epileptogenesis. Our key finding is the demonstration that a transient dose of adenosine delivered locally via a silk-based brain implant can suppress the development of epilepsy long-term in a rat model of progressive epilepsy. It is important to keep in mind that silk is a bioresorbable material; thus, the transient delivery of adenosine via silk, will have long-lasting effects, whereas the silk will gradually be resorbed leaving no bioburdens in the treated brain. To further improve clinically translatability we developed an injectable silk-based gel formulation to provide a high dose of local adenosine. We designed a strategy to separately assess the therapeutic effects of a transient 'burst' of adenosine release and those from a sustained longer-term release of a lower dose. Clinically, an injectable formulation of adenosine releasing silk will offer a more versatile and less invasive alternative for antiepileptogenic treatment.

The translational impact of our studies is high. Focal adenosine augmentation could easily be implemented as a safe treatment option for patients with early signs of epilepsy as well as those at risk of developing epilepsy. For example, therapeutic adenosine augmentation could be used as a preventative measure following severe TBI or following epilepsy surgery, which bears an inherent risk of secondary epileptogenesis. An important aspect is our finding that the transient increase of adenosine provides long-lasting benefit. Adenosine kinase inhibitors have been in the pharmaceutical drug development pipeline as adenosine augmenting drugs, mostly during the time span of 2000 - 2005; however, those systemically active drugs never made it to the clinic due to unacceptable side effects (mostly sedation, and liver toxicity after long-term use) of chronic drug dosing. Our findings that transient focal adenosine delivery prevents epileptogenesis are a major achievement in harnessing the advantages of adenosine therapy while avoiding negative side effects associated with systemic or chronic drug dosing.